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Antimicrobial Susceptibility Patterns of Common and Unusual Species of Enterococci Causing Infections in the United States

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We collected 705 isolates of enterococci (1 per patient) from cultures of a variety of anatomic sites from patients at eight tertiary-care hospitals in six geographic regions of the United States. A total of 632 (90%) *Enterococcus faecalis*, 58 (8%) *E. faecium*, 5 *E. gallinarum*, 4 *E. avium*, 3 *E. casseliflavus*, 1 *E. raffinosus*, and 1 *E. hirae* isolate and 1 biochemical variant of *E. faecalis* were identified; 606 (86%) of these isolates were associated with clinical infections. The most common sites of isolation were the urinary tract (402 [57%]), nonsurgical wounds (94 [13%]), the bloodstream (74 [10%]), and surgical wounds (62 [9%]). High-level resistance to gentamicin or streptomycin or both was detected in 265 (38%) of the isolates. We identified two *E. faecalis* isolates resistant to vancomycin (MICs, 32 and 128 μg/ml) and 11 β-lactamase-producing *E. faecalis* isolates. *E. faecium* isolates were significantly more resistant than *E. faecalis* isolates to penicillin, ampicillin, piperacillin, imipenem, and ciprofloxacin ($P < 0.001$). The MICs for the 15 non-*E. faecalis*, non-*E. faecium* enterococci indicated variable resistance to ciprofloxacin and the penicillins. Antimicrobial susceptibility patterns vary among species of enterococci, and these organisms, while commonly resistant to high-level aminoglycosides, can also acquire resistance to vancomycin or the ability to produce β-lactamase. Because of these diverse antimicrobial resistance mechanisms, successful treatment and control of enterococcal infections with current antimicrobial agents are becoming increasingly difficult.

Enterococci are considered a part of the normal flora of the bowel, genital tract, and anterior urethrae of humans (15, 23). Although enterococci have been considered of relatively low virulence, these organisms can cause serious infections, including endocarditis (12, 24, 26, 27). In addition, urinary tract infections are commonly caused by enterococci, particularly among hospitalized patients (24, 26). The National Nosocomial Infections Surveillance System reported enterococci to be the second most common pathogen associated with nosocomial urinary tract infections, causing 16% of approximately 35,000 nosocomial urinary tract infections reported during 1986 to 1989 (40).

Although 12 species within the genus *Enterococcus* are recognized (4, 5, 8, 16, 27, 42), *Enterococcus faecalis* accounts for approximately 90% of enterococci reported from clinical sources. However, most studies have been limited to enterococcal bloodstream infections and have not used the most recent methods for species identification (7, 36). Species identification of enterococci may be useful both as an epidemiologic tool in the investigation of outbreaks of nosocomial infections and, because antimicrobial susceptibilities may vary by species, for clinical decisions about therapy (21, 25, 27, 39).

We conducted a study (i) to characterize the species of enterococci causing infections among patients receiving care at tertiary-care centers in diverse geographic locations in the United States, (ii) to determine their patterns of antimicrobial resistance, including high-level aminoglycoside resistance, vancomycin resistance, penicillin or ampicillin resistance, and β-lactamase production, and (iii) to characterize the differences in antimicrobial susceptibility among the newly described enterococcal species.

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MATERIALS AND METHODS

The study was conducted at eight tertiary-care hospitals with ≥250 beds, located in six geographic regions throughout the United States. From July 1988 through April 1989, investigators from each hospital were asked to submit all enterococcal strains isolated (one per patient) from clinical sites to the Centers for Disease Control (CDC). Data collected for each patient included whether the isolate was associated with infection or colonization according to CDC definitions (10); the age and sex of the patient and the date of admission to the hospital (if an inpatient was involved); the date and source of the culture; and whether the infection or colonization was polymicrobial. If the source of the isolate

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was the urinary tract, information regarding instrumentation of the urinary tract before onset of infection was requested.

Organisms. Isolates were inoculated on Trypticase soy agar slants (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and sent to the CDC for species identification and antimicrobial susceptibility testing.

Identification of *Enterococcus* species. Isolates were identified to genus and species level by previously described conventional tests based on Gram stain, hemolysis, pigment, gas production, motility, and selected physiologic tests to determine growth in 6.5% NaCl and at 10°C, the ability to blacken bile esculin medium, and the ability to hydrolyze 1-pyrrolidonyl-β-naphthylamide (7). Tellurite reduction was used to differentiate *Enterococcus solitarius* from lactose-negative *E. faecalis* (7).

Antimicrobial susceptibility testing. All isolates identified as enterococci were tested by standard broth microdilution tests with Mueller-Hinton broth (Difco, Detroit, Mich.), following recommendations for cation supplementation by the National Committee for Clinical Laboratory Standards (30, 31). Inocula were prepared from overnight growth on a blood agar plate by suspending the growth in Mueller-Hinton broth. The inoculum was adjusted to a 1.0 McFarland standard for inoculation of the broth microdilution plates with the MIC 2000 mechanical inoculator (Dynatech Laboratories, Inc., Chantilly, Va.). The final inoculum in the broth test was approximately 3×10^5 to 5×10^5 CFU/ml. All plates were incubated for 18 to 20 h at 35°C in ambient air.

All strains were tested for susceptibility to ampicillin, ciprofloxacin, gentamicin, imipenem, kanamycin, penicillin, piperacillin, streptomycin, teicoplanin, and vancomycin. In addition, β-lactamase-producing strains were tested for susceptibility to ampicillin-sulbactam (2:1) and amoxicillin-clavulanate (2:1). Aminoglycosides were tested with serial twofold dilutions of 128 to 0.5 μg/ml and at concentrations of 2,000 and 500 μg/ml.

High-level aminoglycoside resistance was defined as an MIC of ≥2,000 μg/ml. Penicillin, ampicillin, piperacillin, and teicoplanin resistance was defined as an MIC of ≥16 μg/ml. Vancomycin and imipenem resistance was defined as an MIC of ≥8 μg/ml, while ciprofloxacin resistance was defined as an MIC of ≥2 μg/ml. Except for the aminoglycosides, these definitions of resistance represent both intermediate-moderately susceptible and resistant categories as defined by the National Committee for Clinical Laboratory Standards (31).

β-Lactamase testing. All isolates were screened for β-lactamase production with a nitrocefin (Glaxo, Inc., Research Triangle Park, N.C.) solution at a concentration of 500 μg/ml. Growth was suspended in 0.05 ml of the solution and read for a color change for up to 60 min (33).

Data analysis. Data for enterococcal species were compared by using the chi-square test or two-tailed Fisher's exact test as appropriate. The software package Epi Info (CDC, Atlanta, Ga.) was used for data entry and statistical analysis.

RESULTS

A total of 705 isolates were received during the 10-month study period. Of these, 632 (90%) were *E. faecalis*, 58 (8%) were *E. faecium*, and 15 (2%) were other *Enterococcus* spp. (five were *E. gallinarum*, four were *E. avium*, three were *E. casseliflavus*, one was *E. raffinosus*, one was *E. hirae*, and one was a biochemical variant of *E. faecalis*).

The distribution by site of isolation for the 705 isolates included 402 (57.0%) from the urinary tract; 94 (13.3%) from nonsurgical wounds (including decubitus ulcers); 74 (10.5%) from the bloodstream (including two from patients with endocarditis); 62 (8.8%) from surgical wounds; and 73 (10.4%) from miscellaneous sites, including the biliary tract, peritoneum, respiratory tract, and cerebrospinal fluid. There were no significant differences in the distribution of species by site.

TABLE 1. High-level gentamicin and streptomycin resistance among 705 clinical isolates of enterococci, by species

Drug	No. (%) of resistant strains			
	E. faecalis (n = 632)	*E. faecium* (n = 58)	Other *Enterococcus* spp. (n = 15)	Total (n = 705)
Streptomycin	88 (13.9)	20 (32.8)	1	109 (15.5)
Gentamicin	68 (10.7)	1 (1.7)	1	70 (9.9)
Both streptomycin and gentamicin	78 (12.3)	8 (13.8)	0	86 (12.2)

Patient characteristics. Of the 705 persons from whom isolates were obtained, 349 (49.5%) were females, 342 (48.5%) were males, and 14 (2.0%) did not have their sex recorded; 84% were inpatients, and 96% were >18 years of age. The median age of the patients was 62 years (range, 11 months to 97 years).

Clinical infections were reported for 605 (86%) isolates, and colonization was reported for 50 (7.1%) isolates; the infection and colonization data were incomplete for the remaining 50 isolates. The infections were polymicrobial in 385 (55%) of the cases, including 22 (30%) of 74 bloodstream infections.

Risk factors. A total of 339 (84%) of the urinary tract isolates were associated with infection; 75% of the urine cultures contained $>10^5$ CFU/ml. A total of 144 (43%) patients with urinary tract infections had undergone instrumentation of their urinary tracts (mostly urinary catheterization) within the 48 h preceding the urine culture. A total of 46 (61%) of the 72 patients with bloodstream infections had a peripheral, central, or umbilical catheter in place within the 48 h preceding the blood culture. Among the 574 inpatients, the median duration between date of culture and date of admission to the hospital was 7 days (range, 1 to 285 days).

High-level aminoglycoside resistance of enterococci. High-level resistance to aminoglycosides was detected in 268 (38%) of the isolates. The prevalence among the hospitals of high-level gentamicin resistance ranged from 1% (1 of 95 isolates) to 70% (14 of 20 isolates).

The frequency of high-level aminoglycoside resistance by species ranged from 50% among the 58 *E. faecium* isolates to 13% among the 15 non-*E. faecalis* enterococcal isolates ($P >$ 0.05 [chi-square test for trend]). Nine patterns of high-level aminoglycoside resistance were identified among the 268 isolates. The most common patterns of high-level resistance were gentamicin-streptomycin-kanamycin (32%; 85 of 268 isolates), streptomycin-kanamycin (31%; 84 of 268 isolates), and gentamicin-kanamycin (26%; 69 of 268 isolates). High-level resistance to a single aminoglycoside was unusual and was detected only for streptomycin (22 isolates) and kanamycin (three isolates). High-level resistance to gentamicin was always linked with high-level resistance to kanamycin (156 of 156 isolates). The resistances most frequently detected by clinical laboratories were to streptomycin and gentamicin. The results for these two drugs alone and in

TABLE 2. Ranges of MICs of nonaminoglycoside antimicrobial agents for 14 enterococcal isolates (non-*E. faecalis*, non-*E. faecium*) by species

Organism (*n*)	MIC range (μg/ml)						
	Penicillin	Ampicillin	Piperacillin	Imipenem	Vancomycin	Teicoplanin	Ciprofloxacin
E. gallinarum (5)	1–4	1–2	16–>16	1–2	4–8	0.5–2	2–>8
E. avium (4)	1–2	0.5–1	16–>16	0.5–1	0.5–1	0.5	1–2
E. casseliflavus (3)	0.5–4	0.5–2	8–>16	0.5–4	2–8	0.5–1	0.5–4
E. raffinosus (1)	32	16	>16	8	1.0	0.5	1.0
E. hirae (1)	2	2	>16	2	0.5	0.25	0.5

combination are shown in Table 1. Of note, 45% (70 of 156) of the isolates with high-level resistance to gentamicin were susceptible to streptomycin.

Characteristics of unusual isolates of enterococcal spp. The 15 isolates of unusual species of enterococci were from various anatomic sites of patients at several hospitals. High-level resistance to aminoglycosides was detected in two isolates, *E. raffinosus* and *E. gallinarum*, the former having high-level resistance to gentamicin. For the other antimicrobial agents tested, ranges of MICs by species are given in Table 2. The small numbers of these isolates limit generalizations about MICs in comparing them with the *E. faecalis* or *E. faecium* isolates (Table 3). However, resistance to penicillins and imipenem was detected in the *E. raffinosus* isolate. There appeared to be more resistance to piperacillin in the non-*E. faecalis*, non-*E. faecium* group than in the *E. faecalis* and *E. faecium* isolates tested. For the strains of *E. gallinarum* tested, the MICs of ciprofloxacin were slightly higher than those for the other species tested.

Differences in antimicrobial resistance among *E. faecalis* and *E. faecium* isolates. Overall, resistance to penicillin, ampicillin, piperacillin, ciprofloxacin, and imipenem among strains of *E. faecium* was significantly higher than among strains of *E. faecalis* (Table 3).

Penicillin and ampicillin. *E. faecium* was significantly more likely than *E. faecalis* to be resistant to penicillin or ampicillin (MICs, ≥16 μg/ml) (for penicillin, 34 of 58 *E. faecium* isolates [59%] versus 0 of 632 *E. faecalis* isolates [$P < 0.0001$]; for ampicillin, 24 of 58 *E. faecium* isolates [41%] versus 0 of 632 *E. faecalis* isolates [$P < 0.0001$]). The MICs of penicillin were one to two dilutions higher than the MICs of ampicillin for 97% of the isolates tested.

β-Lactamase-producing isolates. Of the 705 isolates tested, 11 (1.6%) were β-lactamase producers. All were *E. faecalis*, and all demonstrated an inoculum effect with both penicillin and ampicillin. Although MICs for these isolates were in the susceptible range (penicillin, 4 to 8 μg/ml and ampicillin, 1 to 4 μg/ml at low levels of inoculum [10^5 CFU/ml]), increasing the inoculum to 10^7 CFU/ml increased the MICs of penicillin and ampicillin to >64 μg/ml. All β-lactamase-producing isolates were susceptible in vitro to ampicillin-sulbactam (MICs, 1.0/0.5 to 2/1.0 μg/ml, respectively) and amoxicillin-clavulanic acid (MICs, 0.5/0.25 to 1.0/0.5 μg/ml, respectively). A total of 10 of 11 isolates demonstrated high-level resistance to streptomycin and kanamycin; all 11 isolates demonstrated high levels of resistance to gentamicin. A total of 10 of the 11 β-lactamase-producing isolates were collected from patients at a single hospital during an 8-week period. The sites of isolation were the urinary tract (seven), wounds (two), and the respiratory tract (one); nine of these β-lactamase-producing isolates were associated with infection.

Piperacillin. *E. faecium* was also more likely than *E. faecalis* to be resistant to piperacillin, with a breakpoint of ≥16 μg/ml (42 of 58 [72%] versus 0 of 632; $P < 0.001$). The MICs of penicillin for several strains of *E. faecium* (8 of 58 [14%]) were ≤8 μg/ml but were >16 μg/ml with piperacillin. Since the MIC results for piperacillin tend to cluster around the breakpoint, the clinical significance of this finding is unknown.

Imipenem. The MICs of imipenem ranged from ≤0.25 to 32 μg/ml. *E. faecium* strains were significantly more likely to be resistant to imipenem (MIC, ≥8) than *E. faecalis* (33 of 58 [57%] versus 0 of 632 [$P < 0.001$]). All penicillin- and ampicillin-resistant *E. faecium* strains were also resistant to imipenem.

Ciprofloxacin. The MICs for *E. faecalis* ranged from 0.06 to >8 μg/ml; however, although the MICs for the majority of the strains were at the breakpoints (1 to 4 μg/ml), only 1% of the strains were fully resistant to ciprofloxacin with MICs of ≥4 μg/ml. For the *E. faecium* isolates, the MICs ranged from 0.25 to >8 μg/ml, but the MICs for only 5% of the strains were ≥4 μg/ml.

Vancomycin and teicoplanin. Only 0.3% (2 of 705) of the isolates were resistant to vancomycin (MICs of 32 and 128 μg/ml). Both strains were *E. faecalis* and were isolated, respectively, from the urine and a nonsurgical wound of patients at the same institution. Both isolates demonstrated high-level resistance to gentamicin (but not streptomycin) and did not produce β-lactamase. All isolates, including both

TABLE 3. MIC_{50}, MIC_{90} and range of MICs[a] of nonaminoglycoside antimicrobial agents for 691 *E. faecalis* and *E. faecium* isolates

Species and drug	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)
E. faecalis (*n* = 633[b])			
Penicillin	2	4	0.12–8
Ampicillin	1	2	0.12–4
Piperacillin	4	8	0.12–16
Imipenem	1	2	0.12–8
Vancomycin	2	4	0.5–128
Teicoplanin	0.25	0.5	0.12–4
Ciprofloxacin	1	2	≤0.25–>8
E. faecium (*n* = 58)			
Penicillin	32	>128	≤0.25–128
Ampicillin	8	64	≤0.12–64
Piperacillin	>16	>16	2–>16
Imipenem	16	32	≤0.25–16
Vancomycin	1	2	0.5–8
Teicoplanin	0.5	1	≤0.12–1
Ciprofloxacin	4	4	≤0.25–>8

[a] MIC_{50}, MIC for 50% of strains tested; MIC_{90}, MIC for 90% of strains tested.
[b] Includes an *E. faecalis* variant.

vancomycin-resistant strains, were susceptible to teicoplanin (MIC range, 0.12 to 4 μg/ml).

DISCUSSION

The spectrum of infection associated with the enterococcal isolates in this study was diverse and occurred in a patient population that was characterized as elderly (median age of 64), inpatient, and evenly distributed by sex. Almost 98% of the enterococci were *E. faecalis* or *E. faecium*, comparable to the distribution of species found in recent studies of clinical isolates (11, 21, 22, 46). Serious enterococcal infections (i.e., in the bloodstream and cerebrospinal fluid) accounted for approximately 11% of the study isolates. The urinary tract was the most common site of infection, which often occurred after instrumentation of the patient's urinary tract.

Enterococci were isolated from the bloodstream of 74 study patients, thus causing approximately 11% of the infections. These enterococcal bloodstream infections usually occurred in patients without endocarditis and were often polymicrobial (29%).

The overall prevalence of high-level resistance to any aminoglycoside among the study isolates was 38%. The frequencies of high-level resistance to the individual aminoglycosides were similar to those recently reported for enterococci isolated from patients in Ohio and Boston, Mass. (21, 36). High-level resistance usually occurred in combinations of more than one aminoglycoside. Several patterns of high-level resistance in enterococci were identified, but none were specific to a single institution or species.

Enterococci with high-level gentamicin resistance were common (22.1%), detected at all institutions, and always associated with high-level resistance to tobramycin and kanamycin, suggesting the presence of the *aph2″-aac6′* gene combination. It is important to emphasize that certain strains with high-level gentamicin resistance did not have high-level resistance to streptomycin. A total of 45% of the 156 isolates with high-level gentamicin resistance were not highly resistant to streptomycin; therefore, cell-wall inhibitors in combination with streptomycin may be useful in the treatment of serious infections due to these organisms. Streptomycin susceptibility in 25 to 33% of other highly gentamicin-resistant enterococcal strains has been reported elsewhere (29). Since amikacin is sometimes considered for combination therapy, it is important to note that amikacin cannot be used to determine *E. faecalis* susceptibility to amikacin-penicillin synergy (27, 38). Kanamycin has been shown to more accurately predict amikacin-penicillin synergy than does amikacin (38). Similarly, gentamicin should be used to predict tobramycin-penicillin synergy (38).

Species identification of isolates enabled us to assess species-specific antimicrobial resistance characteristics. *E. faecium* isolates were significantly more likely to be resistant to the penicillins, imipenem, and ciprofloxacin than non-β-lactamase-producing isolates of *E. faecalis*. Penicillin- and ampicillin-resistant isolates were found only among non-*E. faecalis* species of enterococci. This finding is consistent with previous reports of non-β-lactamase-producing penicillin-resistant enterococci (1, 3, 6, 37, 39).

Like the penicillins, imipenem is not bactericidal against enterococci and probably should not be considered for use as a single agent in treatment of serious enterococcal infections (2, 19, 32, 41). All *E. faecalis* isolates (including β-lactamase producers) were susceptible to imipenem, in contrast to *E. faecium* isolates.

Ciprofloxacin has been used successfully for treatment of enterococcal urinary tract infections (13, 48), and we found 97% of *E. faecalis* isolates to be susceptible to ciprofloxacin. However, the in vitro bactericidal effect of ciprofloxacin is highly inoculum dependent; when used alone or combined with gentamicin in the treatment of experimental enterococcal endocarditis in rabbits, ciprofloxacin was significantly less effective than penicillin alone or penicillin combined with gentamicin (9, 49). Therefore, even when isolates are shown to be susceptible by MIC, ciprofloxacin may not be the best alternative for the treatment of serious enterococcal infections.

The overall prevalence of vancomycin resistance among enterococcal isolates in our study was low (<1%). Both isolates had what would be characterized as low-level resistance to vancomycin on the basis of the MICs of vancomycin for them (8 μg/ml < MICs < 256 μg/ml) and their susceptibility to teicoplanin (17, 18, 20, 28, 43–45, 47).

We identified 11 *E. faecalis* isolates as β-lactamase producers, all demonstrating an inoculum effect with β-lactam antibiotics. Production of β-lactamase in these strains is usually plasmid mediated and frequently is not detected by routine disk susceptibility or dilution testing. Each isolate appeared to be susceptible to penicillins by routine MIC testing, but increasing the inoculum to 10^7 CFU/ml resulted in MICs of ≥64 μg/ml for both penicillin and ampicillin. For infections due to β-lactamase-producing enterococci, ampicillin-sulbactam or vancomycin may be the initial antimicrobial of choice (27).

Although the overall prevalence (1.5%) of β-lactamase producers was low, the finding of a previously undetected cluster of 10 isolates associated with clinical infections in a single hospital suggests that screening of enterococcal isolates for β-lactamase production should be practiced more routinely in clinical laboratories by using a nitrocefin-based test, in particular on those isolates with high-level aminoglycoside resistance that are recovered from normally sterile body sites. β-Lactamase-producing enterococci have been reported from at least nine cities in the United States, including an outbreak of β-lactamase-producing *E. faecalis* in an infant-toddler surgical ward in Boston, Mass. (14, 28, 34, 35).

In summary, this investigation permitted the determination of the prevalence of antimicrobial resistance among enterococci causing a wide spectrum of diseases among patients in diverse geographic locations. Identification of enterococcal isolates to the species level in the clinical microbiology laboratory is useful because it can help predict patterns of antimicrobial susceptibility, particularly to penicillins. In serious clinical diseases (e.g., bloodstream infections or meningitis), identification to the species level and determination of high-level aminoglycoside resistance to gentamicin and streptomycin should be strongly encouraged because of the differences in antimicrobial susceptibilities between *E. faecium* and *E. faecalis*. Except for resistance to penicillins, the non-*E. faecalis*, non-*E. faecium* isolates appeared similar to *E. faecalis* isolates in their MICs. β-Lactamase-producing enterococcal strains may remain undetected, because most clinical microbiology laboratories do not routinely screen enterococcal isolates for the production of β-lactamase and routine susceptibility tests often do not detect it. The problem of treatment and control of enterococcal infections is underscored by the high prevalence of nosocomial isolates and their ability to acquire resistance to the limited number of useful antimicrobial agents available in the treatment of enterococcal infections.

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